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Acute toxic and hepatopancreas syndrome caused by Chlopyrifos ethyl to black tiger shrimp (*Penaeus monodon*) and white shrimp (*Litopenaeus vannamei*) in Mekong River Delta of Vietnam

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Abstract: Acute toxicity (96h) and chronic toxic (60 days early life stage) tests were conducted in laboratory to determine the LC_{50} and hepatopancreas syndrome of liver and gill of Chlopyrifos ethyl, an organo phosphorus pesticide commonly used in Vietnam, to two major brackish water shrimps *Penaeus monodon and Litopenaeus vannamei* of Mekong River Delta to ensure the relevant of Chlopyrifos ethyl use with early mortality symptom (EMS) of shrimps in Asia countries and the safety standard for water environment. The results indicate that the acute median lethal concentration (LC_{50}) of Chlopyrifos ethyl to adults of *Penaeus monodon* and *Litopenaeus vannamei*, young age of *Litopenaeus vannamei* and *Penaeus monodon* is 0.1412; 0.1197; 0.0042 and 0.0041 mgl^{-1} respectively. It means adults of both shrimps are more tolerant to Chlopyrifos ethyl than young ones (about 30 times) while the difference of tolerant between to tested species are not clearly recognized. Surgical histopathological assessment of hepatopancreas syndrome necrotizing pancreatitis in Chlorpyrifos ethyl showed exposure at low concentrations, shrimp in hepatopancreas samples collected at 10 and 20 day after treatment. Expression of the transformation is phenomenal concentration of blood cells around the liver pancreas and some changes in the structure of the hepatopancreas tube. When exposed at higher concentrations (80% or higher value of LC), the majority of experimental shrimp died within 10 days after exposure.

Keywords: Chlorpyrifos ethyl; acute toxic; syndrome hepatopancreas, *Penaeus monodon*; *Litopenaeus vannamei*

1. Introduction

The Symptom of early mortality (EMS) enclosed with histopathology of liver and gill of two brackish water shrimps *Penaeus monodon* and *Litopenaeus vannamei* is

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threshing shrimp culture in Vietnam and Asia countries as well. Up to date, there has been no reason determined precisely, but the contamination of pesticide is considered as one of the direct or indirect potential hazards. Chlorpyrifos ethyl, a high toxic insecticide ranked to 2nd toxic group by WHO standard is commonly used in Vietnam to control various insect pests in paddy rice and upland crops. Due to high effective against a wide range of arthropods and insect pests including Coleoptera, Diptera, Homoptera and Lepidoptera species, Chlorpyrifos is high toxic to the nervous system and is also transformed inside animals to chlorpyrifos-oxon and 3, 5, 6-trichloro-2-pyridinol (TCP) both of which are many times more toxic to the nervous system than chlorpyrifos itself (Chambers, J.E., Forsyth, C.S. and Chamber, H.W., 1989). Due to its lipophilic nature, fish are able to absorb and bio-concentrate chlorpyrifos to moderate or high levels and variable bio-concentration factors (BCF) between 100-5100 have been reported (Racked, K.D., 1993). They enter water bodies through intentional application, run-off from farms, aerial drift and accidental and illegal release (Zelikoff, J.T., 1994). In Vietnam, there were 164 registered products containing Chlorpyrifos (Vietnam, 2014)].

Due to high dose and frequency usage, the contamination of Chlopyrifos ethyl to water source is considered closely relevant. Meanwhile, the shortage of specific study on level of acute toxic and chronic symptom caused by Chlorpyrifos ethyl to the above shrimp species is constrain preventing the examination and treating measurement of EMS. This research aim to provide scientific date to ensure the potential relevant of Chlorpyrifos ethyl to EMS and to assist promulgating country's standard for MRL of Chlorpyrifos ethyl in shrimp culture water.

2. Materials and methods

2.1. Materials

- Young *Penaeus monodon* and *Litopenaeus vannamei* (at post 15 and 12 respectively) supplied by local breeding agency were transported to the laboratory of Cantho University in a portable well-aerated white polythene bag containing 2% salt water from the breeding pool and transferred into composite tank $2m^3$ in 3-5 days. They were health checked before chemical treatment to confirm zero infection of liver pancreatic necrosis and then switch to the other tank containing salty water at 2% concentration (measured by refract meter), using Na_2CO_3 with volume $40gm^{-3}$ to raise the pH to about 10-20 mgl^{-1} and maintaining continuous aeration. Temperature stability culture 26-28^{0}C. Water pH, temperature and salinity remain stablely during the experiment time to ensure deviation of temperature less than 1^{0}C and salt concentrations less than 0.2% within 24 hours. During this period, the shrimps were fed with

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pelleted diet containing 35% crude protein twice per day at 5% body weight. Also, the water in the glass aquaria was changed once every two days (Reish, D.L. and Oshida, P.S., 1987). Tests were undertaken with both young and adult shrimps grown up from young one (at 30 days age after rearing from post 15 and post 12 as above).

- Water: the sea brine is removed from the salt production base, adjust pH to 8.0 and salinity to 2%.
- Chemical: Technical grade containing 97% ai

2.2. Methodology

2.2.1. Acute bioassays: LC_{50} 96-h static bioassays were conducted in the laboratory following the methods of Spragne, J.B., (1975) and APHA; AWWA and WEF (1985). The acute concentrations for chlorpyrifos-ethyl were determined by two steps:

- Step 1: preliminary test: conducted with 8 treatment, including 7 concentrations of Chlorpyrifos ethyl and untreated check (UTC). Tested concentrations were determined by selecting one reference concentration which is an average of LC_{50} value of Chlorpyrifos ethyl published for aquatic animals by Canada water quality guidelines for the protection of aquatic life (about 0.0268) plus with 3 higher and 3 lower levels of 10, 100 and 1000 times. Hence, the concentration range was 0.0000268; 0.000268; 0.00268; 0.0268; 0.268; 2.68; 26.8 and UTC.
- Step 2: Final test: basing on preliminary results, the lowest concentration of Chlorpyrifos ethyl which provided approaching 50% lethal will be selected for starting concentration of final test. Six higher concentration next to starting concentration were developed by x2; x4; x8; x 16; x32 and x64 times, and UTC.

Each concentration was replicated three times. Post stocking density was 30 individuals/ 5lit glass tank and adult stocking density was 20 units/ tank. The shrimp stock was moved to 25L of de-chlorinated tap water in the glass aquaria and stand for 30 minutes before introducing to test.

Survival and mortality were recorded from 1 to 6, 8, 16, 24, 72 and 96 hours. Shrimps were considered dead when the opercula movement ceased and there was no response to gentle probing. Identify LC_{50} values by using Probit software BioStat denotes the relationship between pesticide concentration and mortality rate.

2.2.2. Sub-lethal bioassay and histological procedures
Based on 96-h LC50 value, the concentrations for chronic study was 10%, 20%; 40%; 60%, 80%, 100%, 120% LC_{50} as USEPA guideline (Oladimeji, A.A and Ologunmeta, R.T., 1987) and UTC. Each treatment was replicated by 3 times. Number of shrimps for each replicate was maintained as the same acute test. Test solutions were changed every 12hrs to ensure stable initial concentration less than 10% degradation during testing time (Flores, V., Galan, M., and Sales, D., 1980). Swollen abdomen was sampled at every 10 days within 60 days after pesticide handling. Each tank surgery three individuals. For histopathological analysis, shrimp sampled were injected with AFA Davidson's fixative, processed, and stained with hematoxylin and eosinphloxine (H&E) using routine histological methods described by Lightner (1996). The histological sections were analyzed by light microscopy (x10, x40 and x100 for typical lesions of acute hepatopancreatic necrosis syndrome or any abnormal lesions in the HP magnifications.

3. Results and discussion

3.1. Determination of LC_{50} – 96h values

3.1.1. Preliminary experiments: Preliminary results indicated that adults of *Litopenaeus vannamei* were killed at the starting concentration ($0.0000268mgl^{-1}$) of chlorpyrifos but at low mortality. When rising tested concentration to 0.000268 and $0.00268mgl^{-1}$, the effect of chemical to that target shrimp was not significantly increased. Where as *Penaeus monodon* at both ages and young stage of *Litopenaeus vannamei* were began to die at the concentration of $0.00268mgl^{-1}$ (Table 1).

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Table 1: Mortality of *Penaeus monodon and Litopenaeus vannamei* **caused by Chlorpyrifos ethyl in priliminary tests**

(Can Tho University Lab., 2013)

Treatment ID	Concentration *(mgl^{-1})*	Mortality in 96h *(%)*			
		P/ P15	P/ adult	L/ P12	L/ adult
T1	0.0000268	0.00	0.00	0.00	4.44
T2	0.000268	0.00	0.00	0.00	3.33
T3	0.00268	21.59	3.57	22.62	8.89
T4	0.0268	100.00	25.00	70.24	88.00
T5	0.268	100.00	100.00	100.00	91.11
T6	2.68	100.00	100.00	100.00	96.67
T7	26.8	100.00	100.00	100.00	100
T8	UTC	0	0	0	0

Legent: T: treatment; P: Penaeus monodon; L: Litopenaeus vannamei; P12 and P15: post 12 and post 15; UTC: Untreated check

3.1.2. Preliminary experiments: Based on data of preliminary test, $0.00268mgl^{-1}$ was selected as the starting concentration for final test. Six of next concentrations were determined by multiple this concentration by 2; 4; 8; 16; 32 and 64 times. It was indicated that both *Penaeus monodon and Litopenaeus vannamei at all tested ages* were began to die at the first concentration of final test. Young shrimps showed more sensitive with chlorpyrifos at the same concentration than adult ones. When raising concentration by 8 times of starting one (means 0.02144mg $liter^{-1}$), young shrimps of both species were completely died when chemical killed only haft of adults at that concentration. From the concentration of 0,04288 $liter^{-1}$ and further, almost adult shrimps were killed (Table 2).

Table 2: Mortality of *Penaeus monodon and Litopenaeus vannamei* **caused by Chlorpyrifos ethyl in preliminary tests**

(Can Tho University Lab., 2013)

Treatment ID	**Concentration** *(mgl^{-1})*	Mortality in 96h *(%)*			
		P/ P15	**P/ adult**	**L/ P12**	**L/ adult**
T1	0.00268	17.78	6.67	21.11	6.67
T2	0.00536	63.33	9.94	61.1	13.33
T3	0.01072	92.22	20.00	100	33.33
T4	0.02144	100	55.23	100	66.67
T5	0.04288	100	96.67	100	100
T6	0.08576	100	100	100	100
T7	0.17152	100	100	100	100
T8	UTC	0	1.67	0	0

Legent: T: treatment; P: Penaeus monodon; L: Litopenaeus vannamei; P12 and P15: post 12 and post 15; UTC: Untreated check

3.1.3. Determination of LC50

Wirth data collected from the final test, the probit lines are developed by Biostat sofware in Figure 1, 2, 3 and 4 to analyse the correlation between log of chemical concentration and mortality rate of young and adult of *Penaeus monodon* and *Litopenaeus vannamei* respectively, hence the LC50 value is automaticaly determined by the Biostat sofware (Figure 3). It is indicated that the highest LC50 value of Chlopyrifos ethyl is recorded with adults of *Penaeus monodon* (0.1412mgl^{-1}), next to adults of *Litopenaeus vannamei*, young age of *Litopenaeus vannamei* and *Penaeus monodon* with the values of 0.1197; 0.0042 and 0.0041 mgl^{-1} respectively. It means adults of both shrimps are more tolerant to Chlopyrifos ethyl than young ones (about 30 times) while the difference of tolerant between two tested species are not clearly recognized (Table 3). This study also indicated that both brackish water shrimp species currently cultivated in Mekong River Delta of Vietnam is more tolerant to Chlorpyrifos ethyl than other tested aquatic

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animals which can be died 50% at the concentration of 0.00268mgl^{-1} in average only.

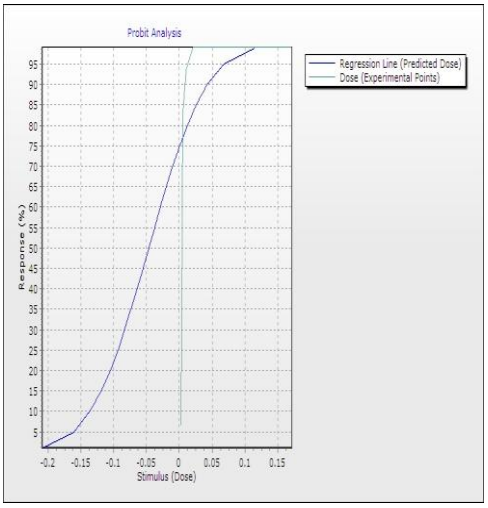

Figure 1: Probit line for determining LC_{50} of Chlopyrifos ethyl to young age of *Penaeus monodon*

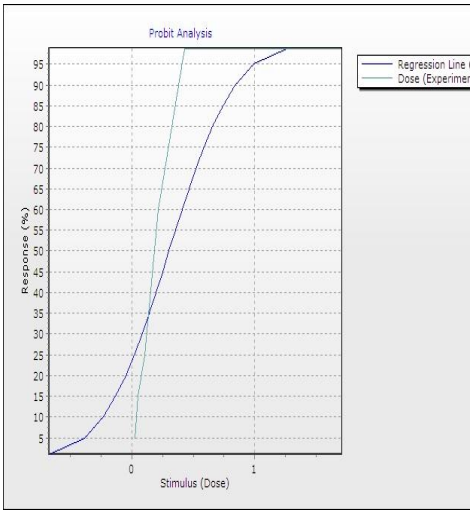

Figure 2: Probit line for determining LC_{50} of Chlopyrifos ethyl to adult of *Penaeus monodon*

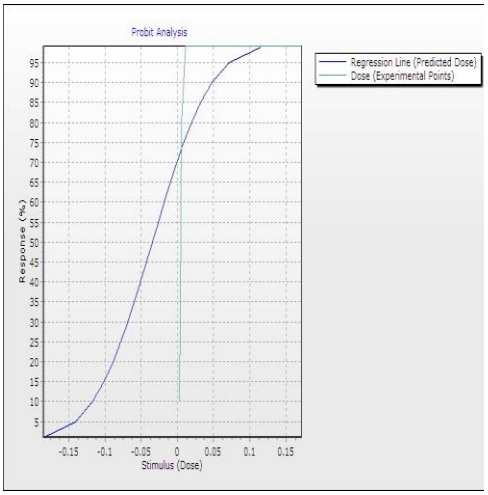

Figure 3: Probit line for determining LC_{50} of Chlopyrifos ethyl to young age of *Litopenaeus vannamei*

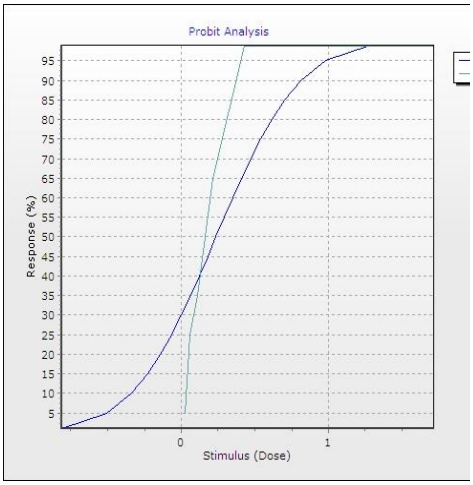

Figure 4: Probit line for determining LC_{50} of Chlopyrifos ethyl to adult of *Litopenaeus vannamei*

Table 3: The Biostat software analysis to determine LC50 value of Chlopyrifos ethyl to *Penaeus monodon* and *Litopenaeus vannamei*

Species/age	Determined mortality (%)	Probit (Y)	Log10[Dose (Stimulus)]	Dose (Stimulus)
Penaeus monodon/ Young	50	5	-2.3876	0.0041
Penaeus monodon/ Adult	50	5	-0.8501	0.1412
Litopenaeus vannamei/ Young	50	5	-2.3773	0.0042
Litopenaeus vannamei/ Adult	50	5	-0.9218	0.1197

3.2. Histology of gills and liver

The experiment was arranged with healthy shrimps (no liver disease pancreatic necrosis) tested by molecular biological methods. Sampling was conducted at 6 times, 10 days/ time from pesticide treatment. The samples obtained from the experiments were evaluated liver syndrome pancreatic necrosis by histopathological methods. Results are summarized in Table 4.

Table 4: Surgical histopathological assessment of hepatopancreas syndrome necrotizing pancreatitis in Chlorpyrifos ethyl

Sampling / Dose	1st	2nd	3rd	4th	5th	6th
10% LC_{50}	(-)	(-)	(-)	(-)	(-)	(-)
20% LC_{50}	(+)	(-)	(-)	(-)	(-)	(-)
40% LC_{50}	(-)	(-)	(-)	(-)	(-)	(-)
60% LC_{50}	(+)	(-)	(-)	(-)	(-)	(-)
80% LC_{50}	(+)	(-)	(-)	(-)	(-)	(-)
100% LC_{50}	(*)					
120% LC_{50}	(*)					
UTC	(-)	(-)	(-)	(-)	(-)	(-)

*Legend***:** *(+) abnormalities sign on liver and gill; (-) No abnormalities sign, (*) no sample due to mortality*

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The histopathology specimens collected form UTC tank showed no signs of liver necrosis, as well as any abnormal expression through repeated sampling. Results are shown in Figure 5. Figure 5 showed a normal pancreas liver with the presence of pancreatic cell types B, R, F in hepatic duct, the normal division of E cells and no presence of the infectious pathogens (viruses, bacteria). The above results demonstrate the stability of experiment condition during layout.

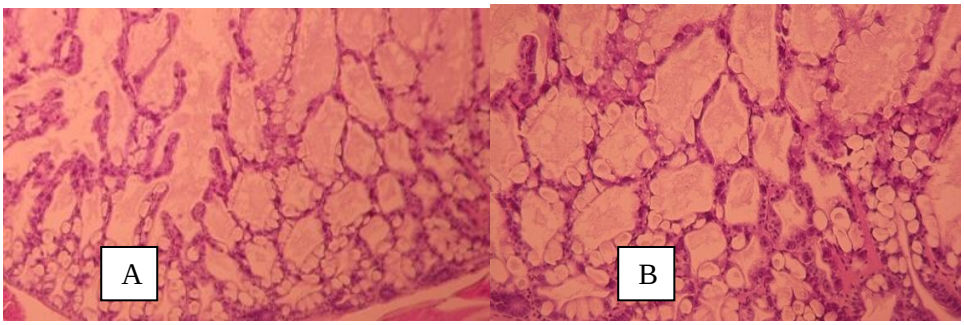

Figure 5: Histopathology shrimp hepatopancreas in the untreated groups (A: at 10days after treatment, B: at 40 days)

At the same time, it was also noted undetectable necrosis signs and any signs of liver disease when treated shrimps at the concentration equivalent with 10 and 40% LC_{50} value. However, several specimens collected from treatment 2 and 4 (20 and 60% LC_{50} value) can be detected some histopathological changes of the liver pancreas. Dynamic of histological treatments are shown in Figure 6, 7, 8, 9.

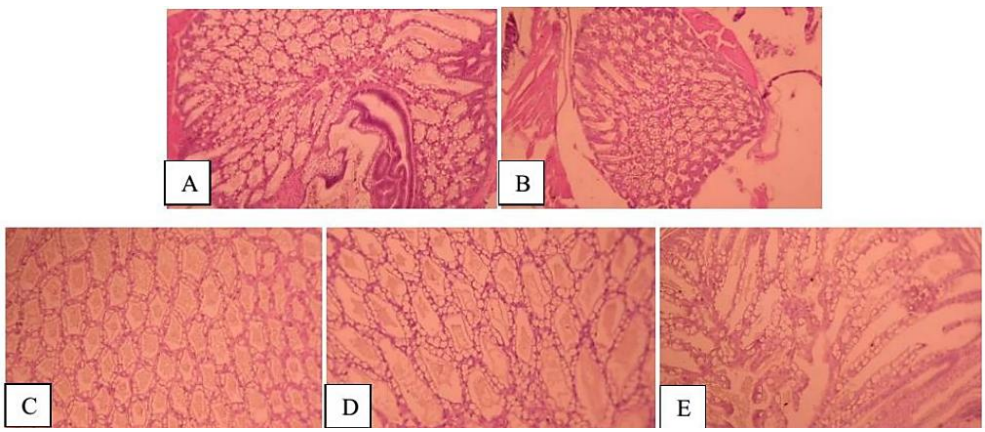

Figure 6. Histopathology shrimp hepatopancreas when treated with Chlopyrifos Ethyl at 10%LC_{50} value sampled at 10 days (A); 20 days (B); 30 days (C); 40 days (D) and 50 days (E). No sign of abnormality recognized

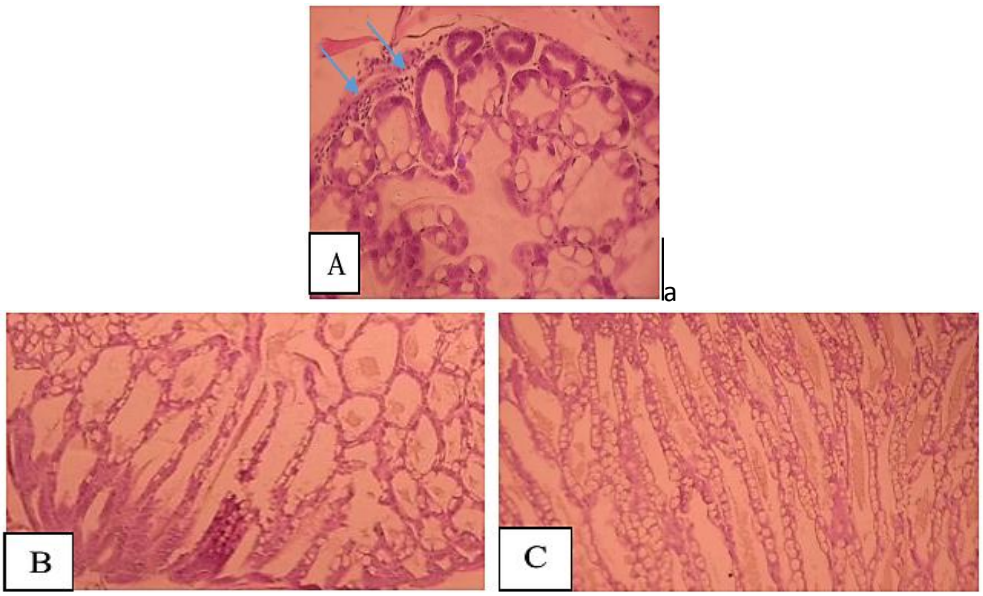

Figure 7. Histopathology shrimp hepatopancreas when treated with Chlopyrifos Ethyl at 20%LC_{50} value sampled at 10 days (A); 50 days (B); 60 days (C). A showed the recognition of blood cell concentrated around the hepatopancreas (arrow)

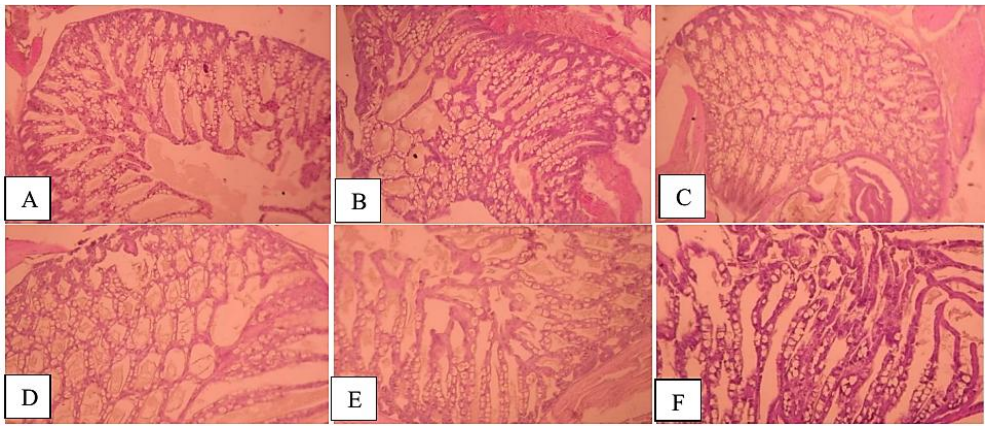

Figure 8. Histopathology shrimp hepatopancreas when treated with Chlopyrifos Ethyl at 40%LC_{50} value sampled at 10 days (A); 20 days (B); 30 days (C); 40 days (D); 50 days (E) and 60 days (F). No sign of abnormality recognized

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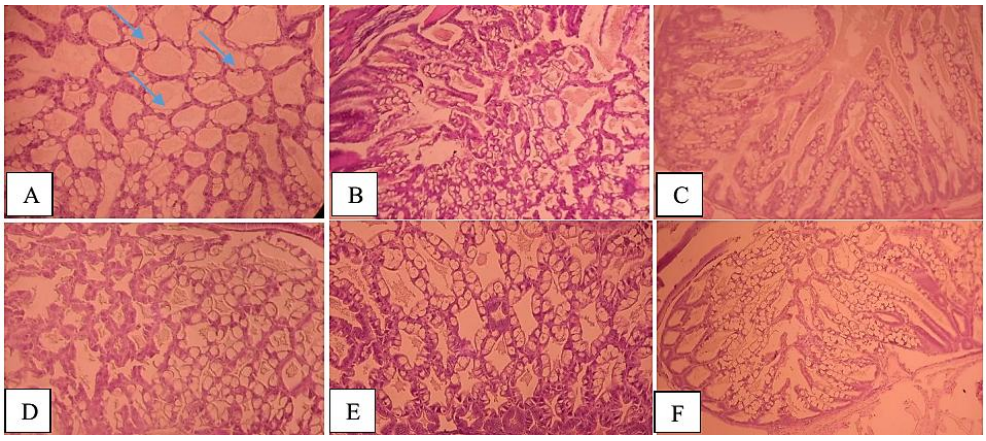

Figure 9. Histopathology shrimp hepatopancreas when treated with Chlopyrifos Ethyl at 60%LC_{50} value sampled at 10 days (A); 20 days (B); 30 days (C); 40 days (D); 50 days (E) and 60 days (F). A showed hepatopancreatic tubes constricted, reducing B cells, R, F (arrows)

The abortion indicated that thought there were changes on the hepatopancreas of shrimp collected from treatments 2 (20% of LC_{50} value) at 10days after treatment, only detecting the concentration of blood cells around liver pancreas (Figure 7), no change in the structure of the pancreatic duct liver. However, when observing the specimen collected from treatments 4 (60% of LC_{50} value) at 10 days, there were some abnormal signs of hepatopancreatic tubes constricted enclosed with the decrease of number of cells B, R, F (Figure 9). Where as there was no change with other treatments (1 and 3) and other observation time. When contacting with the concentration of 100% and 120% of LC_{50} value, almost shrimps died during 10days after treatment. No sample can be collected for surgery.

4. Conclusions

1. The acute median lethal concentration (LC_{50}) of Chlopyrifos ethyl to adults of *Penaeus monodon* and *Litopenaeus vannamei* is, young age of *Litopenaeus vannamei* and *Penaeus monodon* is 0.1412; 0.1197; 0.0042 and 0.0041 mgl^{-1} respectively. It means adults of both shrimps are more tolerant to Chlopyrifos ethyl than young ones (about 30 times) while the difference of tolerant between to tested species are not clearly recognized.
2. When exposure at low concentrations, there was an expression of the transformation of blood cells of both shrimps around the liver pancreas and some changes in the structure of the hepatopancreas tube. When exposed at higher concentrations (80% or higher value of LC), the majority of experimental shrimp died within 10 days after exposure.

3. Thought there is an abnormal of blood cells, it causes to several individuals only and does not cause shrimp death. This findings allow to conclude that the mortality of shrimps is mainly caused by acute toxic of Chlorpyrifos ethyl when exposing at high concentration.

Referrences

1. American Public Health Association, *American Water Works Association (AWWA) and Water Environment Federation (WEF)*, Standard Methods for the examination of Water and Waste water, 18th Edition; Washington, D.C (1985)
2. Canada water quality guidelines for the protection of aquatic life
3. Chambers, J.E., Forsyth, C.S. and Chamber, H.W. *Bioactivation and detoxification of organophosphorus insecticides in rat brains*". In Caldwell, J., Hutson D.H., and Paulson. G.D. Intermediary xenobiotic metabolism: Methodology, mechanisms and significance", Basingstoke, U.K: Taylor and Francis. (1989). pp 99-115.
4. Flores, V., Galan, M., and Sales, D. *Contaminacion de las agues de la Bahia de Gadiz (IV).* Ensayos de biodegradabilidad con dodecilsulfato sodico. Ingenieria Quimica, 131: (1980) 81-111.
5. Lightner, D.V. (1996). *A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp*. World Aquaculture Society, Baton Rouge, LA
6. Oladimeji, A.A and Ologunmeta, R.T. *Toxicity of water-borne lead to Tilapia niloticus. Nig. J.* Applied Fish. Hydrobiol. 2: (1987) 19-24.
7. Racke, K.D. *Environmental fate of Chlorpyrifos*. Review of environmental contamination and toxicology 131: (1993) 1-150.
8. Reish, D.L. and Oshida, P.S. *Manual of methods in aquatic environment research Part 10. Short term static bioassay*. FAO fisheries technical paper 247, FAO Rome (1987) pp 1- 62.
9. Spragne, J.B. *Measurement of pollution toxicity to fish*: In: Bioassay methods for acute toxicity Water Research, 3: (1975) 125-127.
10. Vietnam, *List of pesticide permitted to use in Vietnam* (2014).
11. Zelikoff, J.T. *Fish Immunotoxicology*. In Immunotoxicology and Immunopharmacology (eds. J.H. Dean, M.I. Luster, and A.E. Munson) Raven Press, New York. (1994) pp. 71-95.

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